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Source and Transport of Human Enteric Viruses in Deep Municipal Water Supply Wells

Kenneth R. Bradbury,[†,*] Mark A. Borchardt,[‡] Madeline Gotkowitz,[†] Susan K. Spencer,[‡] Jun Zhu,[§] and Randall J. Hunt[⊥]

[†]Wisconsin Geological and Natural History Survey, University of Wisconsin−Extension, 3817 Mineral Point Road, Madison, Wisconsin 53705, United States

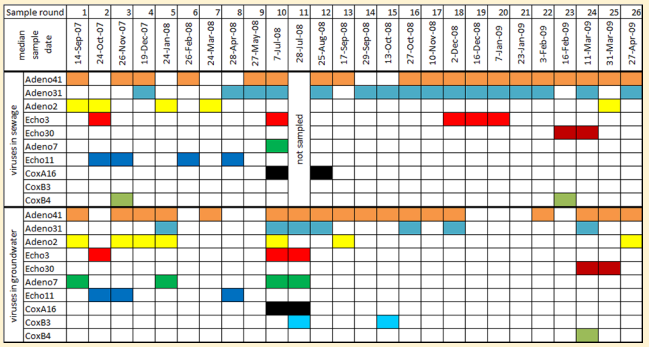
[‡]USDA−Agricultural Research Service, 2615 Yellowstone Drive, Marshfield, Wisconsin 54449, United States

[§]Department of Statistics and Department of Entomology, University of Wisconsin−Madison, 1300 University Avenue, Madison, Wisconsin 53706, United States

[⊥]U.S. Geological Survey, Wisconsin Water Science Center, 8505 Research Way, Middleton, Wisconsin 53562, United States

Supporting Information

ABSTRACT: Until recently, few water utilities or researchers were aware of possible virus presence in deep aquifers and wells. During 2008 and 2009 we collected a time series of virus samples from six deep municipal water-supply wells. The wells range in depth from approximately 220 to 300 m and draw water from a sandstone aquifer. Three of these wells draw water from beneath a regional aquitard, and three draw water from both above and below the aquitard. We also sampled a local lake and untreated sewage as potential virus sources. Viruses were detected up to 61% of the time in each well sampled, and many groundwater samples were positive for virus infectivity. Lake samples contained viruses over 75% of the time. Virus concentrations and serotypes observed varied markedly with time in all samples. Sewage samples were all extremely high in virus concentration. Virus serotypes detected in sewage and groundwater were temporally correlated, suggesting very rapid virus transport, on the order of weeks, from the source(s) to wells. Adenovirus and enterovirus levels in the wells were associated with precipitation events. The most likely source of the viruses in the wells was leakage of untreated sewage from sanitary sewer pipes.



INTRODUCTION

Recent studies have demonstrated widespread occurrence of human enteric viruses in domestic and municipal wells in the United States.[1−4] The U.S. Environmental Protection Agency (USEPA) estimates that at some point in time virus contamination will occur in 27% of public water supply wells in the nation.[5] Several viruses are listed on the USEPA's drinking water Contaminant Candidate List, emphasizing that waterborne viruses are a research priority (http://www.epa.gov/safewater/ccl/index.html). Although the vulnerability of groundwater to virus contamination is now recognized, the occurrence of viruses in confined aquifers has rarely been explicitly investigated.

Confined aquifers are major sources of drinking water in many parts of the United States and world. Confined aquifers are bounded by geological formations called aquitards composed of low-permeability materials such as clay or shale. Aquitards restrict vertical water movement to underlying aquifers, and consequently water from confined aquifers is often assumed to be of high sanitary quality and protected from contamination originating at or near the land surface. Recent work suggests such general assumptions are not necessarily warranted. Depth-specific water samples from 91 m in a confined sandstone aquifer in the U.K., bounded by layers of siltstone and mudstone, were positive for coliphages, coliform bacteria, fecal streptococci, and clostridia spores.[6] Cavereau et al.[7] reported in a survey of aquifers in France that samples from confined aquifers were positive for human enteric viruses. In another French study, 4 of 15 samples from a confined aquifer overlain by tens of meters of clay were positive for human adenoviruses, although whether the sampling well was cased into the confined aquifer, eliminating possible water contributions from other strata, was not reported.[8]

During sampling conducted in 2005 and 2006, our team repeatedly detected viruses in two wells cased through a shale aquitard into a confined aquifer in southern Wisconsin, USA.[9] Moreover, five of seven positive samples tested positive for

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culturable viruses, suggesting relatively rapid transport from the virus source to the wells. A likely source of viruses in the wells was leakage of untreated sewage from the local sewer system.[9,10]

The work reported here builds on our previous virus sampling of deep groundwater.[9] Understanding how viruses move from near-surface sources to deep bedrock wells is critical for assessing the magnitude of the virus problem and human health risks, and for developing remedial actions. However, based on the limited sampling to date, it has been difficult to elucidate pathways and mechanisms that deliver viruses to the wells. Given that viruses originate near the land surface, there are four conceptual models of virus transport to the confined aquifer: (1) transport by rapid flow along the well annulus and/or through damaged, deteriorated, or poorly installed grout or breaches in the well casing; (2) transport through the aquitard by porous-media flow; (3) transport by porous-media flow around the edge of the aquitard or through nearby "windows" or breaches in the aquitard; and (4) transport by rapid flow through fractures in the aquitard or through cross-connecting nearby wells.

This paper explores these four hypotheses by using temporal and spatial variability in virus detections and concentrations among municipal wells, surface water, and sewage in a single urban community, Madison, Wisconsin. The wells in Madison are typical of wells now in use throughout the United States. These high-capacity wells are between 5 and 70 years old and were constructed according to accepted well drilling practices, which include grouted well casing to depth. The wells are sampled regularly for coliform bacteria and *E. coli* to meet federal drinking water regulations, and the water is disinfected prior to distribution, but there is no state or federal requirement for virus testing. Our objectives were to measure the temporal coincidence between the virus populations in sewage and the virus populations detected in supply wells, to evaluate sewage as the virus source, and to estimate virus travel times from near surface sources to the deep wells. The relation of precipitation to virus occurrence was also investigated.

EXPERIMENTAL SECTION

Geology, Hydrogeology, and Sampled Wells. Municipal water supplies in Madison, Wisconsin (Figure 1) rely on high-capacity wells completed in confined and unconfined bedrock aquifers.[11] A typical conceptualization of the local hydrostratigraphy (Figure 2) consists of 10−30 m of unlithified materials (till, sand and gravel, or lake sediment) covering a shallow bedrock aquifer composed of sandstone and dolomite. Shale of the Eau Claire Formation forms a regional aquitard and separates the shallow bedrock aquifer from a deep bedrock aquifer. This aquitard is less than about 4 m thick in the Madison area, and is thought to be missing over large areas beneath the central lakes and east of the county. Crystalline rock bounds the bottom of the system. Vertical hydraulic gradients are downward due to a regional cone of depression beneath the Madison metropolitan area.[11] In this situation water and any contaminants in the shallow upper aquifer have the hydraulic potential to move vertically downward into the underlying deep aquifer. Wells are typically cased and grouted through the upper geologic units and consist of open boreholes below the casings. Both near-horizontal and near-vertical fractures are known to be present in the shallow bedrock in the area,[12,13] and such features can represent rapid, "fast" pathways for groundwater movement across the aquitard toward the wells.

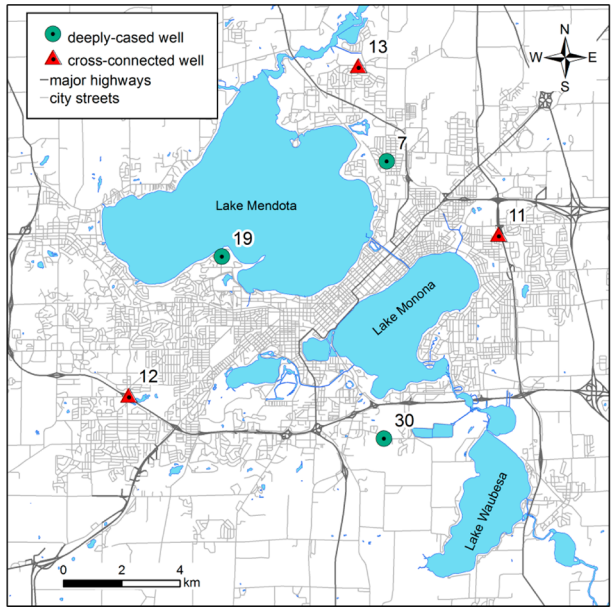

Figure 1. Location of sampled wells in Madison, Wisconsin. Wells 7, 11, 12,13, 19, and 30 are the long-term sampling sites used in this study and all were positive for viruses. Numbers refer to Madison Water Utility well numbers.

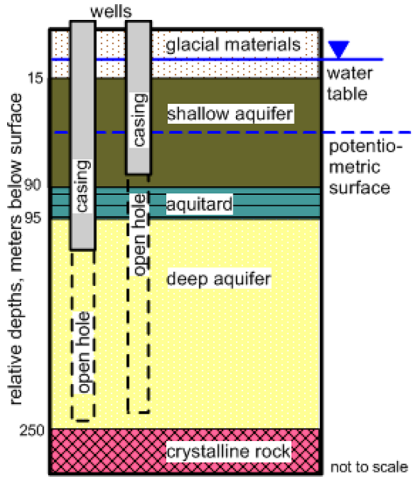

Figure 2. Typical hydrostratigraphy and well construction for the Madison area. The well on the right is a cross-connected well because it is open to both the shallow and deep aquifers.

The sampled wells include three multiaquifer wells (open across the aquitard; wells 11, 12, and 13) and three wells reported to be cased through the aquitard (wells 7, 19, and 30). Construction details of individual wells (Table 1) show the variation in total depth, casing depth, and thickness of the aquitard determined from well construction records. The casings of wells 11, 12, and 13 do not extend through the aquitard, and the open holes connect the upper and lower aquifers. These wells are presumably more vulnerable to contamination than the deeply cased wells. Table 1 also shows the approximate distance from each well to a possible aquitard breach, where the aquitard might be absent, based on unpublished maps at the Wisconsin Geological and Natural History Survey.

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Table 1. Characteristics of Sampled Wells

well no.	year constructed	total depth (m)	casing depth (m)	aquitard depth (m)	aquitard thickness (m)	multiaquifer?	distance to possible aquitard breach (m)
11	1959	229	34	69	2.5	yes	880
12	1957	300	79	123	4.3	yes	4600
13	1959	238	39	70	4.6	yes	650
7	1939	221	72	66	3.1	no	730
19	1970	219	79	75	3.1	no	690
30	2003	244	95	81	3.4	no	690

Virus Sampling. Virus samples were collected every two to four weeks between September 2007 and April 2009 for a total of 26 sampling events. Each sampling "event" required several days to collect well water, lake samples, and sewage samples. Viruses were concentrated by using glass wool filters;[14] where well water pH exceeded 7.5, the pH was adjusted to between 6.5 and 7.0 by using 0.25 M HCl injected ahead of the filter. All well water samples were collected from a sampling tap at the wellheads prior to chlorination while the high-capacity well pumps were running (mean well water sample volume = 984 L, range 280−3180 L, n = 147). Lake samples were collected from shore by using a submersible pump at 1 m depth and pumping lake water through a polypropylene prefilter with nominal pore size of 10 μm (McMaster-Carr, Atlanta, GA) followed by a glass wool filter (mean sample volume = 852 L, range 447−1151 L, n = 25). Field sampling equipment was decontaminated between samples as described by others.[15] Field blanks collected during this project consisted of 19 L of deionized water pumped through a glass wool filter, using decontaminated pump and tubing. All four blanks were negative for viruses, confirming the efficacy of decontamination procedures. Additionally, all negative controls for nucleic acid extraction, PCR amplification, and cell culture procedures in the laboratory were negative throughout the study. Clarified and settled sewage influent (24-h composite) was collected in sterile containers (sample volume = 4 L) at the Madison Metropolitan Sewerage District Nine Springs treatment plant.

Virus Analyses. Filters and sewage samples were transported to the laboratory on ice and processed within 24 to 48 h of sampling. Glass wool filters and prefilters were eluted and the eluate precipitated and concentrated with use of methods described previously.[4,14] The entire 4 L volume of the sewage influent samples was concentrated by using the same secondary concentration procedure as for the filter eluates (i.e., polyethylene glycol precipitation[16]). Final concentrated sample volumes (FCSV) of the filters and sewage influent were stored at −80 °C until analysis.

Samples were analyzed for six virus groups: enteroviruses, adenoviruses, rotavirus, hepatitis A virus (HAV), and norovirus genogroups I and II. Viruses were detected by real-time quantitative PCR (qPCR) or reverse-transcription qPCR (RT-qPCR) and TaqMan probe, using the LightCycler 480 (Roche Diagnostics, Mannheim, Germany). qPCR and RT reaction conditions, primers, probes, standard curve preparation and quality assurance parameters, and the calculations for sample virus concentrations are described by Borchardt et al.[17] qPCR inhibition was measured for every sample and when necessary mitigated by dilution.[17] Controls for each batch of reactions included an extraction negative control (unseeded FCSV), negative controls for the RT and PCR cocktails, and a positive control of known low viral concentration seeded into an FCSV matrix. The prefilter FCSV was analyzed separately for viruses and the results were summed with those of the corresponding glass wool filter to yield the results for the entire sample. Results reported as virus concentration in genomic copies (gc)/L refer to any and all virus types detected in a sample; when a sample was positive for more than one virus type, virus concentration refers to the numbers of each type summed and divided by the sample volume. Virus nondetects were assigned a zero value.

Samples that were qPCR-positive for enterovirus or adenovirus were further evaluated for virus infectivity by cell culture by using three cell lines (BGMK, RD, and Caco-2) or two cell lines (Graham 293 and A549), respectively, as previously described.[17] Infectivity was gauged by two outcome measures: (1) observation of cytopathic effect (CPE) in cultures held 6 weeks, and (2) integrated cell culture-PCR (ICC-PCR) in which a $\geq$ 10-fold increase in virus genomic copies in cell lysates from 2 week or 6 week cultures compared to the initial virus quantity in the FCSV cell culture inoculum was considered infectious. All enterovirus and adenovirus positive samples were identified to serotype by sequencing, using the ABI Prism 3100 Genetic Analyzer and previously described methods.[17]

Statistical Analysis. Regression techniques were used to explore relationships between virus results (concentrations and presence/absence), wells sampled, virus content of sewage, and groundwater recharge events. Statistical analyses were confined to enterovirus and adenovirus data because detections of other enteric viruses were relatively rare. For concentration data, the response variable was the log concentration of a given type of virus in the groundwater. For virus presence/absence, logistic regression used the presence and absence of a virus as the response variable, and hypothesis testing used χ^2 tests. Analysis of covariance (ANCOVA) compares trend over time among different wells. To assess temporal relationships between virus concentrations in wells and in sewage, log concentrations in the sewage within the same sampling event (time lag 0), in the previous event (time lag 1), and in two sampling events previous (time lag 2) were used as predictor variables in the regression. Similarly, to assess the relationship between virus concentrations in the wells and groundwater recharge events, the predictor variables are precipitation on the same day as virus sampling or from between 1 and 7 days previous. All statistics were performed with software package R (R Foundation; http://www.r-project.org/) and the significance level was set at α = 0.05.

RESULTS AND DISCUSSION

Virus Occurrence in Wells, Lake Water, and Sewage. Water samples from wells were positive for viruses in 67 of 147, or 46% of the samples; virus concentrations ranged from nondetectable to 6.3 gc/L, with a mean of 0.7 gc/L and a median of 0.2 gc/L for virus-positive samples. Overall detection

percentages varied through time (Figure 3) from zero in early June 2008 to 100% in March and May of 2009. Summary data,

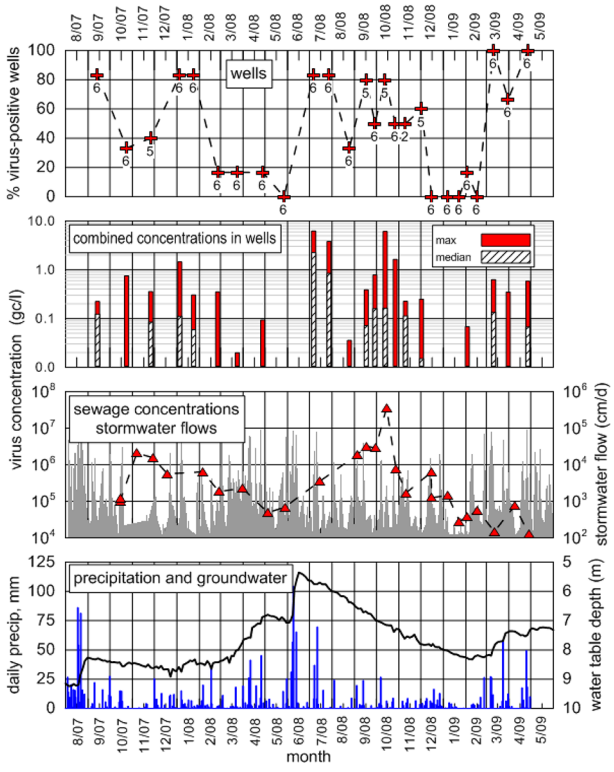

Figure 3. Overall virus detections in wells, monthly precipitation totals, stormwater flows, water table fluctuations, and virus concentrations in sewage influent. Numbers next to well samples indicate the number of wells sampled on that date. Stormwater flow from a large gauged storm sewer discharging to Lake Mendota (USGS site 05427965). Groundwater levels from a water table monitoring well near Sun Prairie, WI.

including statistical results, from all wells are included in the Supporting Information (SI), Section 1. Viruses were detected at least eight times in each of the six wells sampled, but no well was virus-positive in every sampling round (Figure 4). The percentages of groundwater samples positive for viruses ranged from 31% in well 30 to 61% in well 11. Although virus detection and concentration varied markedly with time at each well, the coincidence in detections (Figure 3) and concentration spikes (Figure 4) among all six wells is striking. ANCOVA results (SI, Section 2) show no significant difference in detections or timing between the six wells sampled.

Lake Mendota samples were virus-positive 82% of the time, and concentrations ranged from nondetectable to 530 gc/L, with a mean of 44 gc/L and median of 5 gc/L. Virus concentrations in Lake Mendota peaked at 103 gc/L in October 2008 and decreased during the winter of 2008−2009.

Not surprisingly, sewage influent was extremely high in viruses, with all samples positive and concentrations ranging from 1.3×10^4 gc/L to over 3.6×10^7 gc/L, with a mean of 2.0×10^6 gc/L and median of 1.8×10^5 gc/L (Figure 3). Virus concentrations in sewage peaked in November 2007, declined through May 2008, and then rose to a peak of over 10^7 gc/L in October 2008. Following this peak the sewage virus loads decreased steadily to 10^4 gc/L in May 2009.

Adenovirus 41 was the most frequently identified virus serotype in well water, sewage influent, and lake water (SI,

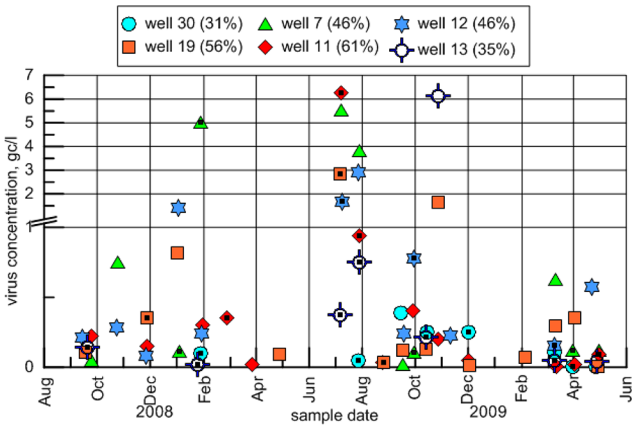

Figure 4. Virus concentrations through time for each of the six wells. Nondetections not shown. A black dot within a symbol indicates that the sample was positive for infectious enterovirus or adenovirus or both. Wells 11, 12, and 13 are multiaquifer; wells 7, 19, and 30 are cased into the deep sandstone. Note the scale change on the *Y* axis in order to improve the visibility of low-concentration samples.

Section 1). Adenovirus 31 was the second most common serotype detected in wells and sewage, but was less common in lake water. Adenovirus 2 was the third most common serotype in wells and sewage and the second most common in lake water. Unlike adenoviruses, enterovirus infections are highly seasonal, occurring in late summer and autumn in Wisconsin,[18] and echoviruses and coxsackieviruses had low detection frequencies in wells, sewage, and lakes. Rotavirus and norovirus genogroups I and II were detected in sewage, but not in the wells (SI, Section 1); no samples were positive for hepatitis A virus.

Some, but not all, well water samples positive for enterovirus or adenovirus were shown to be infective by either cytopathic effect or ICC-qPCR (Figure 4). Enteroviruses and adenoviruses detected in sewage influent samples were always culturable.

Relationships to Geology and Well Construction. Finding human enteric viruses in these wells is consistent with our previous work [9] and shows that even deeply cased municipal wells in confined aquifer settings can be susceptible to pathogen contamination. Casing these deep wells across a regional aquitard neither prevents virus contamination nor even substantially reduces the percentage of virus detections. For the wells reported to be multiaquifer (wells 11, 12, and 13), samples were virus-positive in 34 out of 72 samples, for a detection rate of 47% (SI, Section 1). In reportedly deeply cased wells (7, 19, and 30), the detection rate was 33 detects out of 75 samples, or 44%. The most deeply cased well (well 30) had the lowest percentage of virus detections.

We used linear regression to examine relationships between well construction (total depth, depth of casing), distance to a possible aquitard breach, and virus detections (SI, Section 3). Shallow well casings ($R^2 = 0.11$) and older well age ($R^2 = 0.19$) are weakly correlated to higher virus concentrations, but total well depth or distance to a possible aquitard breach appear unrelated to the well's susceptibility to high virus concentrations. However, the number of wells in this study ($n = 6$) was too small to support any robust statistical tests of these relationships. The elevated virus concentrations measured at wells 7, 11, and 13 might reflect variation in aquifer properties along the deep well bores, with these wells producing a greater

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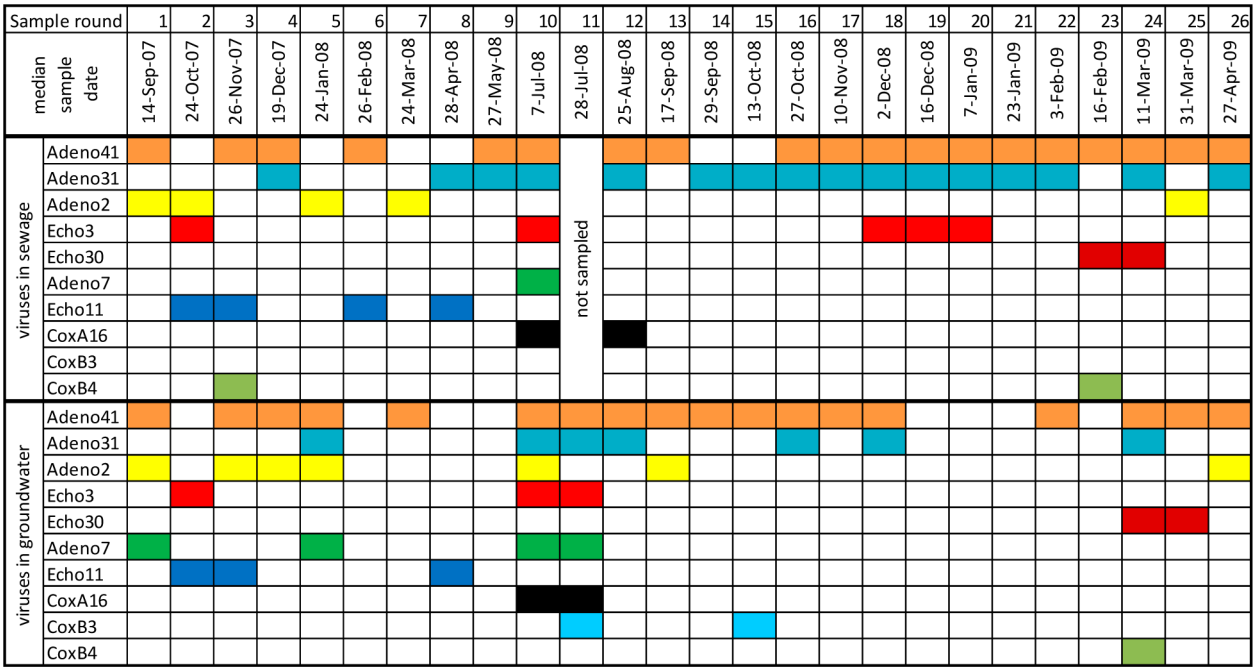
Table 2. Virus Subtypes Detected by Sample Source and Time[a]



[a]Viruses grouped in order of detection frequency. Colors are used to distinguish different serotypes. Numbers and letters refer to virus serotypes: Echo3 (echovirus 3), Adeno41 (Adenovirus 41), etc.

proportion of groundwater from the uppermost portions of the open interval.

Temporal Coincidence of Virus Serotypes between Sewage and Groundwater. The long-term periodic sampling frequency used in the present study was designed, a priori, to use viruses as a tracer to infer the time-of-travel between the suspected virus source, leaking sanitary sewers, and wells. In a human community, different enteric viruses infect and then disappear from the population over time. Such temporal changes in the relative abundance of virus serotypes have been documented for enteroviruses and adenoviruses in wastewater.[19−21] Thus, shed by a variably infected population, the human viruses in wastewater become a "virus signature" for a specific point in time, which then can serve as a tracer for tracking virus movement. For instance, an echovirus 18 appearing in wastewater for the first time in October, but not showing up in well water until December, would suggest a time of travel of two months.

We observed temporal coincidence between virus serotypes present in sewage influent and those serotypes present in groundwater. Table 2 codes each detected serotype by color to make these correlations more visually apparent. The correlations are most obvious with those viruses that have an intermediate to low occurrence; a common virus like Adenovirus 41 is not informative for determining temporal relationships because it is nearly always present in sewage. In contrast, the temporal coincidence of rare viruses is striking. For example, coxsackievirus A16 appears in only two sewage samples in July and August 2008 and it appears in the wells only in July 2008. Adenovirus 31 was not detected in either sewage or wells from September to November 2007, but it appeared in sewage in December 2007 and then in the wells in January 2008. It was not detected in either sample source during February and March 2008, but reappeared in sewage in April 2008 and then again in the wells in July 2008. Adenovirus 2 was present in both sewage and wells in the autumn and early winter of 2007, disappeared from both for 11 consecutive sampling events, and then reappeared in sewage in March 2009 and in the wells during the next sampling event. Echovirus 30 was not detected in any samples until February 2009, when it appeared in sewage during February and March and in wells in both March samples. Echovirus 11 appeared in both sewage and wells in the fall of 2007 and spring of 2008 and then was not detected in either sewage or wells for the remainder of the study. The temporal coincidences are not perfect, though. For example, coxsackievirus B3 was detected during two sampling events in the wells, but never in sewage samples, and echovirus 3 after a seven-sampling-event hiatus appeared again in sewage, but never returned to the wells. These examples might result from issues related to the qPCR analytical sensitivity, qPCR inhibition that was not fully mitigated, the limited sampling used to characterize the sewage source, or to temporal variations in groundwater flow.

Regression analyses support the visual evidence of temporal correlations shown in Table 2 (SI, Section 2). For adenoviruses there is moderate evidence that virus presence (p-value = 0.05) and concentration (p-value = 0.05) are positively related to sewage concentrations during a previous sample event (2−4 weeks earlier). For enteroviruses there is moderate evidence that virus presence (p-value = 0.01) and concentration (p-value = 0.02) are positively related to sewage concentrations during the same sample event.

The presence or absence of identical serotypes in wells and sewage at roughly the same times suggests very rapid transport (days to weeks) between the sanitary sewers and the groundwater system. This is remarkable given the depths of the well casings and that three of the wells were cased through the aquitard into the confined aquifer. Such short times are inconsistent with previous studies[11] in which advective transport times from the source to the deep wells were

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estimated to be 10s to 100s of years. Moreover such short times do not definitively confirm or discount any of the potential flow paths described earlier, although the simultaneous detection of viruses in multiple wells several kilometers apart implies that the failure of well casings is not the only pathway because it would require numerous deep well casings to fail simultaneously. Even so, the correlation between shallow casings and older well age suggests that this pathway could be locally important.

Infiltrating lake water might seem a plausible source for the viruses found in the municipal wells, but three lines of evidence suggest this is unlikely. First, although some viruses (adenovirus 41, adenovirus 2, echovirus 3, echovirus 11) were found in both lakes and wells, other viruses found in wells (adenovirus 7, echovirus 6, coxsackievirus A16) were never found in the lake. Second, deuterium/oxygen-18 relationships (SI, Section 4) suggest that only two wells (7 and 19) receive some proportion of lake-derived water, while all wells contained viruses. Third, virus concentrations in the lakes were generally as low as or lower than virus concentrations in wells. Assuming significant mixing and dilution with virus-free water in the aquifer, the lake virus concentrations were likely too low to account for virus levels measured in the wells.

Sanitary Sewers As a Source of Groundwater Contamination. Several lines of evidence suggest leakage from sanitary sewers beneath Madison was the most likely source of the viruses detected in the municipal wells. First, the raw sewage carries a very high (10^4–10^7 gc/L) virus load, and both the physical characteristics of the sewers (age, location) and visual inspections (video logs showing breaks and root invasions) suggest that they leak. Second, with one exception, all viruses detected in well water were also detected in untreated sewage. Third, virus serotypes identified in the sewage also appear in well water, with significant temporal coincidence between the two, and concentrations of viruses in sewage are temporally correlated to virus concentrations in wells. Fourth, the hydraulic gradients beneath Madison are strongly downward, which would transport viruses downward from the near-surface sewers toward the deep aquifer. In a detailed investigation of the water quality near well 7, Gellasch and others[12] documented the existence of fracture pathways and wastewater indicators in the upper aquifer there.

Relationships between Virus Detections and Recharge Events. Virus detection percentages and concentrations appear to be associated with groundwater recharge events during the study period, in which Madison received unusually high precipitation. Figure 3 shows daily precipitation during the sampling period, typical water table response, typical stormwater flow, virus concentrations in sewage, and virus concentrations and detection frequencies aggregated across wells. In Madison, storm sewers are separate from sanitary sewers and are not considered an appreciable source of viruses to the groundwater system; the stormwater flow in Figure 3 is included to show periods of high surface runoff when higher groundwater recharge was most likely. Rapid decreases in water table depth also indicate recharge events.

Virus concentrations varied through time for individual wells (Figure 4). Eighty percent of the wells were virus-positive the first sampling event, September 2007, and this followed intense rainfall in August (Figure 3) that caused minor flooding. July 2008 increases in virus concentrations and detections followed extreme rainfall events the preceding June, when Madison received 262 mm (10.3 in.) of rainfall during an 8-day period. The March–May 2009 peaks in virus concentrations and detection frequency followed heavy rains during early 2009. All three heavy precipitation periods resulted in episodic recharge events, as indicated by rising water table levels and storm sewer flows (Figure 3). Virus detections and concentrations also peaked in January and February 2008, a season when recharge is rare in Wisconsin because the ground is usually frozen to 0.5–1 m depth. However, the Madison area received record snowfalls in December 2007, and beginning January 1, 2008, diurnal high temperatures became unseasonably warm, melting nearly the entire snowpack and causing high storm sewer flows. Virus transport also might have been enhanced because according to local sewage treatment workers during these heavy precipitation events sections of the sanitary sewers can become surcharged with water and increased leakage of wastewater is very likely. Precipitation events have been associated with waterborne disease outbreaks and with patients seeking medical care for acute gastrointestinal illness.[22−24]

Virus detection varied significantly with time at each well (p-value < 0.0001), but there was no difference in the timing of virus detection among the six wells (p-value = 0.3–0.6; SI, Section 2), suggesting that virus contamination was the result of some regional-scale driver common to the wells sampled. Groundwater recharge from precipitation and snowmelt operates at the regional scale. Enterovirus levels in the wells were correlated with the amount of precipitation that occurred on the same day and 1, 3, 4, and 5 days previous to sampling the wells. Cumulative precipitation measured at all time lags previous to sampling was also correlated with enterovirus levels (with p-values < 0.01). Adenovirus levels in the wells were associated with the precipitation amount on the same day as sampling (p-value < 0.01) and with cumulative precipitation measured one day previous to sampling (p-value = 0.04).

Implications. The simultaneous detection of similar viruses in multiple wells kilometers apart shows that virus presence cannot be attributed to a single point source or a single defective well. Instead, these detections suggest a widely distributed source with at least one fast pathway from the virus source to the wells, and such pathways have been documented at one of the study wells.[12] Although similar studies have not been conducted in confined-aquifer wells in other Wisconsin or Midwestern cities it seems likely that other municipalities with aging sanitary infrastructure and regional cones of depression might have similar groundwater vulnerability to human enteric virus contamination. qPCR-measured viruses in nondisinfected drinking water have been shown to be associated with elevated risk for acute gastrointestinal illness.[17] Moreover, many enteroviruses and adenoviruses detected in the present study were infective. Given that widespread characterization and sampling such as done in this study is not usually feasible, well-maintained disinfection of municipal water systems is likely the most cost-effective way to help ensure public health.

Our results show that virus sampling of municipal wells requires multiple samples over time. Although viruses were found in every well sampled, no well contained detectable viruses on every sample date. Furthermore, on some sample dates no viruses were found in any well, and on other dates every well contained viruses. Single or even quarterly samples from wells will not provide an accurate temporal measure of virus presence in groundwater.

Exfiltration from sanitary sewers had an impact on groundwater quality at significant depths below the water table in this study. Sanitary sewers are a major part of civic

infrastructure and represent a significant potential source of groundwater contamination. Sewer exfiltration, or outward leakage of sewage wastes, represents a potential source of pathogens, toxic chemicals, pharmaceutical compounds, and other materials to the subsurface environment.[25−27] In a study of four unconfined aquifer systems in Wisconsin, Hunt and others[10] found the impact of sewage exfiltration on groundwater and drinking water wells to be variable in time and space, noting that each sampling location that had detections of enteric viruses also had wastewater tracers measured at least one time during their study period. Even with such insights, knowledge about both the quantity of leakage and its consequences for the environment is lacking.[28]

One of the most intriguing findings of this work is the temporal variation and coincidence between virus serotypes in sewage and groundwater. In several instances an occurrence of a "new" virus in sewage is followed within weeks by detection of the same virus in water produced from municipal wells. The implied transport from the sewers to the wells occurs much more rapidly than previous porous-media calculations or modeling have suggested. Hunt et al.[10] attributed such transport to "low yield, fast pathway" conduits in the groundwater system. In the Madison area these fast pathways could include preferential pathways such as fractures, multi-aquifer wells, or poorly grouted well casings.[12] Even when cased through an aquitard into a confined aquifer, wells sited in in urban environments are more vulnerable to virus contamination than often believed.

■ ASSOCIATED CONTENT

Supporting Information

More explanation for (1) well-by-well results, sample distributions, and viruses detected; (2) statistical analyses of relationships between virus detections, sample sites, viruses in sewage, and precipitation events; (3) relationships between virus occurrence, water quality, and well construction; and (4) isotopic results from groundwater and lakes. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: krbradbu@wisc.edu; phone: 608-263-7921; fax: 608-262-8086.

Notes

The authors declare no competing financial interest.

Disclaimer: Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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